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Authors

Lin, Chentao
Luan, Sheng
Tamanoi, Fuyuhiko

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tivity by preventing proteasomal proteolysis of AA-NAT protein. A reasonable hypothetical mechanism underlying the action of cAMP is inhibition of proteasomal targeting by ubiquitination (13).

Cyclic AMP appears to regulate mammalian AA-NAT activity through complementary stimulation of transcription and inhibition of proteasomal proteolysis of AA-NAT protein. Although transcriptional control is not important in all vertebrates (14), inhibition of AA-NAT proteasomal proteolysis may be conserved (13, 15). β -Adrenergic agents may act in a similar manner to control degradation of proteins in other tissues (13, 16).

These findings indicate that proteasomal proteolysis has a role in neural regulation in vertebrates, as in invertebrates (17). Our results indicate that receptor-regulated proteasomal proteolysis can function as a precise, selective, and very rapid neural switch. In the pineal gland, this mechanism regulates the conversion of minute-to-minute changes in environmental input into profound global changes in physiology (18). Such neurally regulated and selective proteasomal proteolysis may play a similarly important role in other aspects of vertebrate physiology and behavior.

REFERENCES AND NOTES

1. A. B. Lerner, J. D. Case, Y. Takahashi, *J. Biol. Chem.* **235**, 1992 (1960); J. Axelrod, *Science* **184**, 1341 (1974); J. Arendt, *Melatonin and the Mammalian Pineal Gland* (Chapman & Hall, London, 1995), pp. 1–273; F. J. Karsch, C. J. I. Woodfill, B. Malpaux, J. E. Robinson, N. L. Wayne, in *The Suprachiasmatic Nucleus: The Mind's Clock*, D. C. Klein, R. Y. Moore, S. M. Reppert, Eds. (Oxford Univ. Press., New York, 1991), pp. 217–230.
2. D. C. Klein and J. L. Weller, *Science* **169**, 1093 (1970); *ibid.* **177**, 532 (1972); H. Illnerova, *Life Sci.* **10**, 955 (1971); S. Binkley, S. E. Macbride, D. C. Klein, C. L. Ralph, *Endocrinology* **96**, 848 (1975); M. D. Rollag and G. D. Niswender, *ibid.* **98**, 482 (1976).
3. D. C. Klein, G. R. Berg, J. L. Weller, W. Glinsmann, *Science* **167**, 1738 (1970); M. Brownstein and J. Axelrod, *ibid.* **184**, 163 (1974); D. C. Klein, N. Schaad, M. A. A. Nambodiri, L. Yu, J. L. Weller, *Biochem. Soc. Trans.* **20**, 299 (1992).
4. Rat AA-NAT (rAA-NAT) antisera (As) 2500 and As2559 were raised against phospho-rAA-NAT_{22–37} (accession number gbU38306) or a mixture of rAA-NAT_{49–59}, rAA-NAT_{72–85}, and rAA-NAT_{89–100}, respectively (19). As2821 was raised against purified, bacterially expressed rAA-NAT_{50–150} fused to measles fusion protein_{288–302} (accession number p41356) [C. Partidos, C. Stanley, M. Steward, *Mol. Immunol.* **29**, 651 (1992)]. rAA-NAT on protein immunoblots (19) was quantitated with a Storm Phosphor-Imager (Molecular Dynamics) or from nonsaturated autoradiographs (X-O-MAT film, Kodak), which were digitized (Microtek Scanmaker II; Adobe Photoshop, version 3.05) and analyzed with NIH Image version 1.57 software. A unit of immunoreactive protein is approximately equal to the signal generated by ~30 pg of bacterially expressed rAANAT, determined with As2559. This is 1% of the signal typically generated by a rat pineal gland obtained in the middle of the night [Zeitgeber time (ZT) 21].
5. M. A. A. Nambodiri, R. Dubbels, D. C. Klein, *Methods Enzymol.* **142**, 583 (1986).

6. T. Deguchi and J. Axelrod, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2547 (1972).
7. J. A. Romero, M. Zatz, J. Axelrod, *ibid.* **72**, 2107 (1975); H. Illnerova, J. Vanecsek, J. Krecsek, L. Wetterberg, J. Sääf, *J. Neurochem.* **32**, 673 (1979).
8. P. H. Roseboom *et al.*, *Endocrinology* **137**, 3033 (1996); M. Bernard *et al.*, *J. Neurochem.* **68**, 213 (1997); D. C. Klein *et al.*, *Recent Prog. Hormone Res.* **52**, 307 (1997).
9. Norepinephrine, isoproterenol, dibutyryl cAMP, and 8-bromocyclic AMP reproducibly increased AA-NAT activity (2) and irAA-NAT (4) in parallel in pineal organ culture (2) and in isolated pinealocytes (20).
10. D. C. Klein, M. J. Buda, C. L. Kapoor, G. Krishna, *Science* **199**, 309 (1978).
11. Cells (~10⁶) were homogenized in 200 μ l of 50 mM Tris (pH 7.5), 500 mM NaCl, 1 mM dithiothreitol (DTT), and 100 μ M phenylmethylsulfonyl fluoride (PMSF); the homogenate was then centrifuged, and AA-NAT was immunoprecipitated with 2 μ l of As2821 (2 hours, 4°C) and protein A-Sepharose (1 hour, 4°C). AA-NAT activity and irAA-NAT are stable under these conditions. AA-NAT was labeled by incubation (1 hour, 30°C, final volume = 20 μ l) with 10 μ l of [³²P]ATP (adenosine triphosphate), 100 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM DTT, 10 mM MgCl₂, and 33 U of protein kinase A (PKA, Promega); radioactive bands were analyzed and quantitated as described (4). This technique is quantitative within the range of AA-NAT values used in these experiments.
12. Rat pinealocytes or pineal glands were treated in experiments similar to those in Fig. 3. Unless otherwise indicated, the concentration of all protease inhibitors was 100 μ M. The following protease inhibitors (targeted class) were ineffective: (serine) PMSF (Sigma), leupeptin (ICN), aprotinin (ICN), *N*-p-tosyl-L-phenylalanine chloromethyl ketone (Calbiochem); (lysosomal) chloroquine (0.2 mM, Sigma); (aspartic) pepstatin (ICN); (metallo) ethylenediamine tetraacetic acid (Sigma); (cysteine) trans-epoxysuccinyl-L-leucylamido (4-guanidino)butane (Sigma), calpeptin (Calbiochem), and α -N-acetyl-leucine-leucine-methioninal (Calbiochem). The following protease or proteasome inhibitors preserved more than 50% of the activity or AA-NAT protein compared with drug alone: calpain inhibitor I and Mg115 [K. L. Rock *et al.*, *Cell* **78**, 761 (1994)], Mg132 (V. J. Palombella, O. J. Rando, A. L. Goldberg, T. Maniatis, *ibid.*, p. 773), Z-leucine-leucine-leucine-vinyl sulfone [M. Bogoy *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 6629 (1997)], lactacystin [G. Fenteany *et al.*, *Science* **268**, 726 (1995)], and β -clastolactacystin [L. R. Dick, L. Cruik-
- shank, F. D. Grenier, S. L. Melandri, R. L. Stein, *J. Biol. Chem.* **271**, 1273 (1997)].
13. Ubiquitination occurs via ϵ -amino groups of lysines in target proteins and is required to target many proteins for proteasomal degradation [L. A. Herschko and A. Ciechanover, *Annu. Rev. Biochem.* **61**, 761 (1992); M. Hochstrasser, *Annu. Rev. Genet.* **30**, 405 (1996); O. Coux, K. Tanaka, A. L. Goldberg, *Annu. Rev. Biochem.* **65**, 801 (1996)]. An NH₂-terminal lysine residue is present in all known vertebrate AA-NAT sequences; this region is important for regulation of AA-NAT activity and irAA-NAT by cAMP and proteasome inhibitors in C6 glioma cells transiently transfected with AA-NAT (J. A. Gastel and D. C. Klein, unpublished observations).
14. S. L. Coon *et al.*, *Science* **270**, 1681 (1995).
15. The rapid suppressive effects of light on melatonin production are evolutionarily conserved: rat (6), human [A. J. Lewy, T. A. Wehr, F. K. Goodwin, D. A. Newsome, S. P. Markey, *Science* **210**, 1267 (1980)], chicken [H. E. Hamm, J. S. Takahashi, M. Menaker, *Brain Res.* **266**, 287 (1983)], and fish [J. Falcon, J. B. Marmillon, B. Claustrat, J. P. Collin, *J. Neurosci.* **9**, 1943 (1989)].
16. P. Costelli *et al.*, *J. Clin. Invest.* **95**, 2367 (1995); W. E. Mitch and A. L. Goldberg, *N. Engl. J. Med.* **335**, 1897 (1996); R. S. Decker *et al.*, *Am. J. Physiol.* **265**, H329 (1993).
17. A. N. Hegde, A. L. Goldberg, J. H. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7436 (1993); A. N. Hegde *et al.*, *Cell* **89**, 115 (1997).
18. L. Tamarkin, C. W. Hollister, N. G. Lefebvre, B. D. Goldman, *Science* **198**, 953 (1977); E. L. Bittman, R. J. Dempsey, F. J. Karsch, *Endocrinology* **113**, 2276 (1983).
19. J. Weller, H. Jaffe, P. H. Roseboom, M. J. Zylka, D. C. Klein, *Brain Res.* **25**, 713 (1996).
20. R. Baler, S. Covington, D. C. Klein, *J. Biol. Chem.* **272**, 6979 (1997).
21. D. W. Wayne, *Biostatistics* (Wiley, New York, ed. 6, 1995), p. 210; *ibid.*, pp. 598–599.
22. We thank S. Coon (NICHD) and R. Baler (NICHD) for technical advice; P. Loh (NICHD), M. Bogoy, and H. Ploegh (Massachusetts Institute of Technology) for helpful discussions; and A. Ciechanover, A. Goldberg, and M. Zatz for use of reagents and equipment. Supported by a Pharmacology Research Associate Training (PRAT) fellowship from NIGMS (J.A.G.).

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Regulation of Flowering Time by *Arabidopsis* Photoreceptors

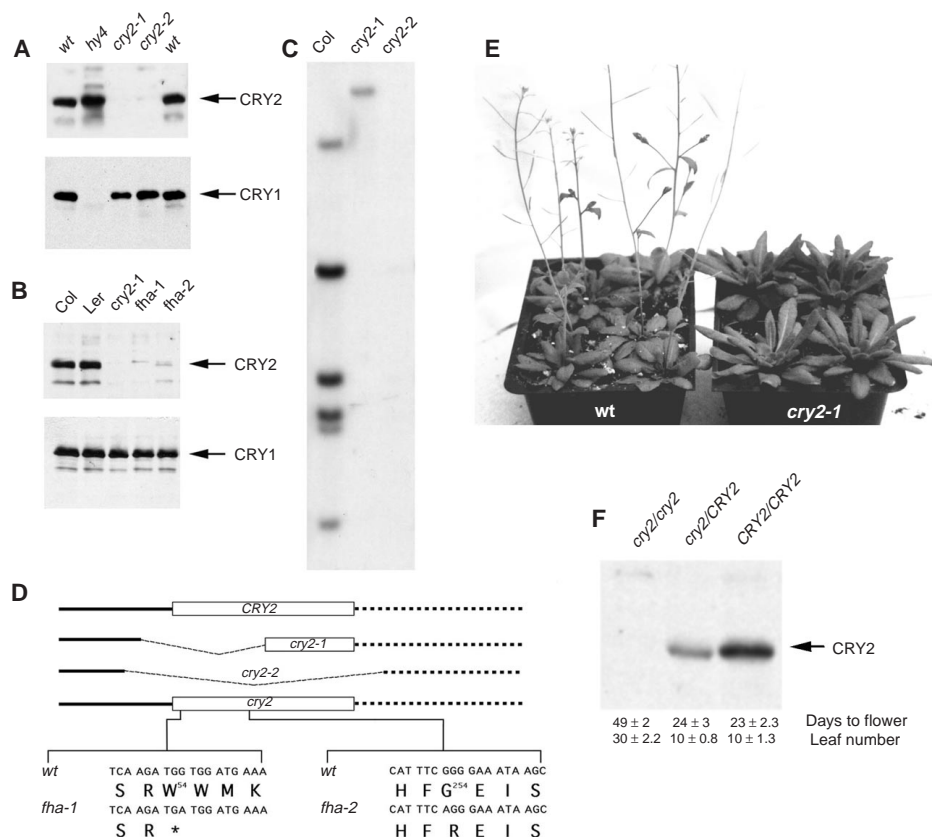
Hongwei Guo,* Hongyun Yang,* Todd C. Mockler, Chentao Lin†

The shift in plants from vegetative growth to floral development is regulated by red–far-red light receptors (phytochromes) and blue–ultraviolet A light receptors (cryptochromes). A mutation in the *Arabidopsis thaliana* CRY2 gene encoding a blue-light receptor apoprotein (CRY2) is allelic to the late-flowering mutant, *fha*. Flowering in *cry2/fha* mutant plants is only incompletely responsive to photoperiod. Cryptochrome 2 (*cry2*) is a positive regulator of the flowering-time gene *CO*, the expression of which is regulated by photoperiod. Analysis of flowering in *cry2* and *phyB* mutants in response to different wavelengths of light indicated that flowering is regulated by the antagonistic actions of *phyB* and *cry2*.

The blue–ultraviolet A (UV-A) light receptors, cryptochromes, and red–far-red light receptors, phytochromes, mediate light-regulated plant growth and development from seed germination to flower ini-

tiation. Phytochrome A (*phyA*), phytochrome B (*phyB*), and cryptochrome 1 (*cry1*) function in both early photomorphogenesis (1–5) and floral induction (6–9). We report that in *Arabidopsis thaliana*, the

Fig. 1. Isolation of *cry2* mutants and characterization of *cry2/fha*. (A) *cry2-1* and *cry2-2* mutants accumulate no CRY2 protein. Samples from *hy4* (*hy4-304*, a *cry1* mutant) *cry2-1*, *cry2-2* mutant (11), and Columbia wild-type (wt) plants were prepared and analyzed, using immunoblot as described (10). The blot was probed with antibody to CRY2 (upper), and was then stripped to remove the anti-CRY2 (10) and re-probed with anti-CRY1 (lower). (B) *fha-1* and *fha-2* mutants accumulate little CRY2 protein. Samples of *cry2-1*, its Columbia parent (Col), *fha-1*, *fha-2*, and their wild-type parent (Ler) were analyzed as in (A). (C) A Southern blot of the genomic DNA of the wild-type (Col) and the *cry2-1* and *cry2-2* mutant plants. Genomic DNA was isolated (28) using the cetyltrimethylammonium bromide method, digested with the restriction enzymes Eco RV and Eco RI, separated on a 1% agarose gel (10 μ g per lane), transferred to a Nylon membrane, and hybridized with 32 P-labeled CRY2 cDNA. (D) The diagram shows mutations in the CRY2 gene (GenBank accession: U43397) of different *cry2/fha* mutant alleles (not to the exact scales). The CRY2 gene is boxed, genomic sequences surrounding CRY2 are represented by solid lines (5') and thick dashed lines (3'), and the thin dashed lines represent deletions. The CRY2 sequences (29) at and flanking mutations found in *fha-1* and *fha-2* are shown in comparison with the corresponding CRY2 sequence of the wild type (Ler). (E) Thirty-four-day-old plants of the *cry2* mutant (*cry2-1*) and wild-type Columbia (wt) grown under continuous white light. (F) An immunoblot showing the absence or presence of CRY2 protein in the homozygous (*cry2/cry2*), heterozygous (*cry2/CRY2*) lines, and wild-type plants (CRY2/CRY2). The flowering time and number of rosette leaves at the emerging of the first flowering buds for each were the averages of a population with more than 20 plants.



blue-light receptor *cry2* (10) plays a major role in floral induction.

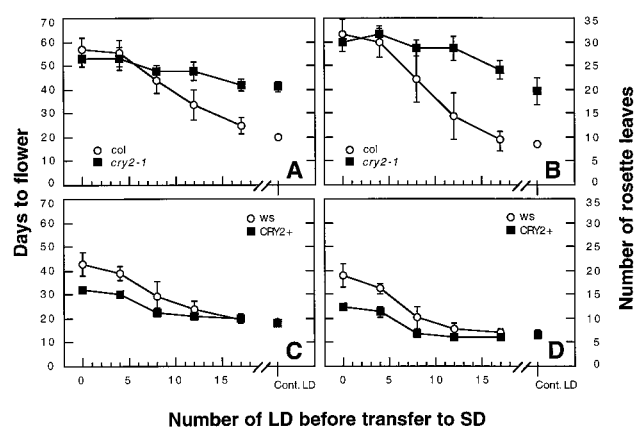
We isolated two *Arabidopsis* mutant alleles that accumulate no CRY2 protein (Fig. 1A) (11). The mutations, *cry2-1* and *cry2-2*, resulted from partial and complete deletion, respectively, of the CRY2 gene (Fig. 1C). Young seedlings of the *cry2* mutants are impaired in blue-light-dependent hypocotyl inhibition and cotyledon opening (10), and *cry2* mutant plants flowered later than normal (Fig. 1, E and F). The late-flowering phenotype of *cry2* mutants is recessive. Plants heterozygous for the *cry2* locus (*cry2/CRY2*) flowered at about the same time as the wild-type plants, although lower levels of CRY2 protein was detected in these plants (Fig. 1F). Because of prolonged vegetative growth, the number of rosette leaves of *cry2* mutant plants was roughly twice normal at flowering (Fig. 1F), as with the late-flowering mutants (12).

Arabidopsis is a facultative long-day plant for which flower initiation is accelerated in long-day (LD) but delayed in short-day (SD)

photoperiods (13, 14). We examined the effect of *cry2* on photoperiod-regulated floral induction (Fig. 2) (15). A similar amount of total irradiation was provided for plants grown under LD and SD to minimize the effect of different day lengths on photosynthesis. The difference between the *cry2* mutant and the wild-type plants in the flowering time (and the number of rosette leaves) was

the greatest for plants grown in LD; this difference diminished when the plants were treated with fewer LD periods (Fig. 2, A and B). Under uninterrupted SD photoperiods, *cry2* mutant plants flowered slightly earlier than the wild-type in SD (Fig. 2, A and B). Therefore, the mutation in the CRY2 gene results in a partial loss of photoperiodic regulation of flowering time. The transgenic

Fig. 2. The photoperiodic response of the *cry2* mutant and CRY2-overexpressing plants. Seeds of *cry2* and the corresponding wild type (Col) (A and B) or the CRY2-overexpressing line H2-9 (CRY2+) and the corresponding wild type (ws) (C and D) were sown on compound soil with a similar density (24 seeds per 3-inch by 5-inch pot), kept at 4°C for 4 days, and grown in LD [18 hours of light ($\sim 100 \mu\text{mol s}^{-1} \text{m}^{-2}$), 6 hours of darkness]. One pot of each line was transferred to SD [9 hours light ($\sim 200 \mu\text{mol s}^{-1} \text{m}^{-2}$), 15 hours darkness] at the time shown on the abscissa or was grown in continuous LD (Cont. LD). "Days to flower" are measured as the days between the date plants were placed under light to the date the first flower bud appeared (A and C). The number of rosette leaves were scored at the day the first flower bud appeared (B and D). The flowering time and the number of rosette leaves shown were the averages of total plants in each pot; the standard deviations are shown.



Department of Molecular, Cell and Developmental Biology, and Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA.

* These authors contributed equally to this work.

† To whom correspondence should be addressed. E-mail: clin@mcd.b.ucla.edu

plants overexpressing CRY2 (10) flowered slightly earlier than the wild type in SD, but at the same time as the wild type in LD (Fig. 2, C and D).

We surveyed other *Arabidopsis* late-flowering mutant lines (*gi*, *co*, *fha*, *fca*, *fd*, *fe*, *ft*, *fua*, *fve*, and *ld*) (12, 16) for the expression of CRY2. One of the late-flowering mutants with reduced sensitivity to photoperiod, *fha* (12, 14), contained mutations in the CRY2 gene. Little CRY2 protein was detected in *fha* mutants (Fig. 1B), although CRY1 expression was unaffected (Fig. 1B). All other late-flowering mutants examined showed normal CRY2 expression. *cry2* and *fha* both flower later, and *cry2-1* failed to complement *fha-1* (17). CRY2 and *FHA* mapped to the same region of chromosome 1 (17). DNA sequence analysis revealed a premature stop codon of CRY2 in *fha-1* (Fig. 1D). The mutation of *fha-2* converts Gly²⁵⁴ of CRY2 to Arg (Fig. 1D). It is unclear why such a missense mutation would result in loss of CRY2 protein in the *fha-2* plants (Fig. 1B), but Gly²⁵⁴ is conserved in both photolyases and cryptochromes (10, 18) and is separated by five residues from another conserved cluster (Thr-Ser-Xaa-Leu-Ser, with Xaa indicating any amino acid). In *Escherichia coli* photolyase, this motif forms polar interactions between the apoprotein backbone and the

phosphate oxygen of the FAD chromophore (19). The change of the aliphatic residue Gly to a basic amino acid Arg at position 254 may have a detrimental effect on the binding of flavin chromophore and thus result in the instability of the mutant protein.

Other *Arabidopsis* late-flowering mutants, *co* and *gi*, also flower late in LD but not in SD (12, 20, 21). The *CONSTANS* (CO) gene encodes a GATA-1-type transcriptional regulator required for the accelerated flowering of *Arabidopsis* in LD (20). The expression of CO itself is regulated by day length; the abundance of CO mRNA is higher in plants grown in LD than in plants grown in SD (20). CO activates the expression of floral meristem identity genes and leads to flower initiation (22). CO has been proposed to be downstream in a signaling pathway from a hypothesized blue light receptor (14). To investigate the relationship of CO and CRY2, we analyzed CO mRNA levels (20) in *cry2* mutant plants grown under different photoperiods (Fig. 3). The expression of the *AP2* gene (23), which is not regulated in response to photoperiod (20), was used as the control (Fig. 3). CO mRNA level detected in the *cry2* mutant plants grown in LD was at least three times lower than that in the corresponding wild-type plants (Fig. 3). CO message amounts in *cry2* mutant grown in SD

were only slightly reduced (Fig. 3), which may explain why *cry2* mutant plants flowered late only in LD (Fig. 2, A and B). Transgenic plants overexpressing CRY2 had CO mRNA levels significantly higher than the wild type in SD but not in LD (Fig. 3), correlating with the flowering time of the transgenic plants (Fig. 2, C and D). These results indicate that *cry2* is a positive regulator of CO in response to photoperiod. *cry2* is apparently not the only photoreceptor regulating CO expression: there was a twofold increase of CO mRNA in the *hyl* mutant impaired in the biosynthesis of the phytochrome chromophore (22), and activity of CO is required for the early-flowering phenotype of *hyl* and *phyB* mutants (20). Thus, cryptochrome 2 and phytochromes appear to function antagonistically in the regulation of CO gene expression.

Blue light (wavelength of ~400 to 500 nm) and red light (~600 to 700 nm) promote and inhibit flowering of *Arabidopsis*, respectively (24, 25), suggesting different functions of phytochromes and cryptochromes in the flowering-time determination. Consistent with previous reports (24, 25), wild-type plants grown under continuous blue light flowered earlier (within 15 days after germination) than plants grown under a similar intensity of red light (more than 30 days after germination) (Fig. 4; red, blue). Considering that blue light promotes flowering and the *cry2* mutant flowered late, it may be expected that *cry2* mutants might flower later than the wild type if grown in continuous blue light. To our surprise, *cry2* mutant plants grown under continuous blue (or red) light flowered at about the same time as the wild type (Fig. 4; red, blue). Because *cry2* mutant plants flowered late under white light, we examined the flowering time of *cry2* mutant plants grown under light containing both blue and red wavelengths. Under this condition, *cry2* mutant plants flowered significantly later than wild-type plants (Fig. 4; red + blue). Thus, the delayed flowering of *cry2* mutant plants under white light can be phenocopied by growing the mutant plants under blue-plus-red light.

Our results suggest that phytochromes mediate the red-light-dependent inhibition of flowering, whereas *cry2* mediates the blue-light-dependent inhibition of phytochrome function. Phytochromes inhibit flowering in the absence of blue-light-dependent CRY2 activity such that red-light-grown wild-type plants flower late. In blue light, wild-type plants flower early, implying either the presence of a blue-light-dependent activator or the absence of a red-light-dependent inhibitor. Normal flowering of *cry2* mutant plants in blue light indicates that the function of *cry2* alone does not promote flowering under blue light. Thus, accelerated flowering of

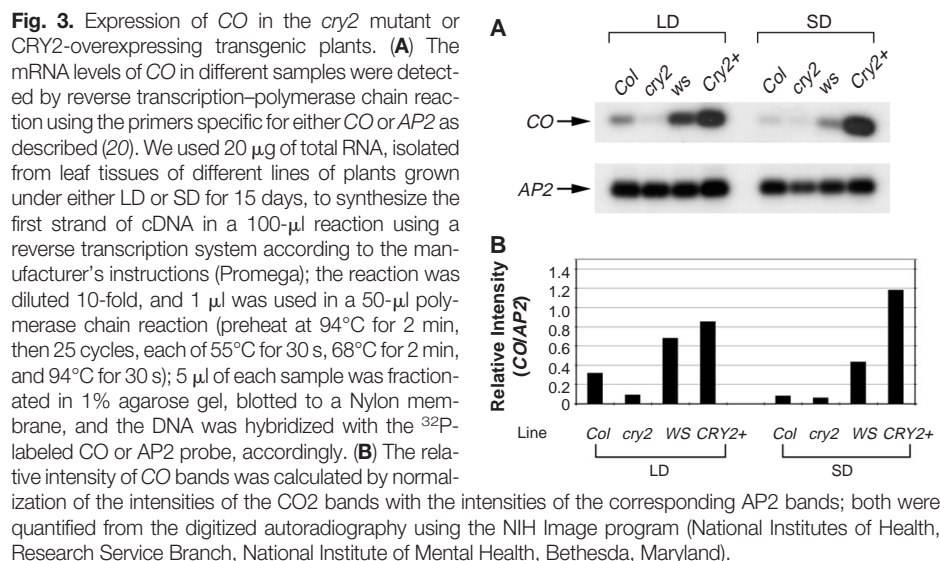
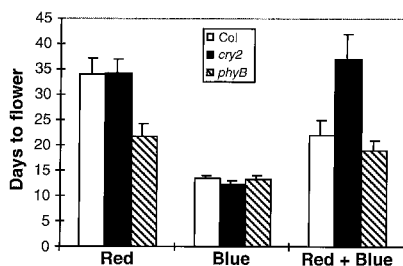


Fig. 4. Effect of different wavelengths of light on the flowering time of *Arabidopsis cry2* and *phyB* mutants. The flowering time was measured as described (Fig. 2) for the Columbia wild type (Col) and the *cry2* (*cry2-1*) and *phyB* (*phyB-9*) mutant plants grown under continuous red (75 to 90 µmol s⁻¹ m⁻²), blue (75 to 85 µmol s⁻¹ m⁻²), or blue-plus-red light (60 to 80 µmol s⁻¹ m⁻², with a ratio of red-light intensity to blue-light intensity of approximately 2 to 3) (10). Means of three independent experiments (individual samples contain more than 20 plants) with slightly different fluence rate from one experiment to another and the standard errors are shown.



wild-type plants in blue light can be at least partially explained by the absence of the activity of the red-light-dependent inhibitors, phytochromes. Under white light or blue-plus-red light, red-light-dependent phytochrome activity and blue-light-dependent cry2 activity function in an antagonistic manner. In these light conditions, cry2 mutant plants flower late because the red-light-dependent phytochrome activity inhibiting floral initiation remains untamed as a result of the lack of the blue-light-dependent cry2 activity in the mutant plants.

We suggest that the function of both phytochromes and cry2 in flowering-time regulation are mediated by CO. The function of phytochromes proposed in our model is consistent with the observation that *Arabidopsis* *hy1* and *hy2* mutants, defective in the biosynthesis of phytochrome chromophore, flower earlier than the wild-type plants (6). It is not clear how many phytochrome species are involved in mediating red-light-dependent inhibition of flowering, although phyA is probably not associated with the flowering inhibition because the phyA mutant does not flower early (6). *phyB* mutant plants flower earlier than the wild-type plants grown under white light (6, 7), an effect mediated by CO (20). Thus, phyB could be one of the phytochromes that mediates red-light-dependent inhibition of flowering (4). Indeed, the early-flowering phenotype of *phyB* is dependent on red light (Fig. 4). In blue light, however, *phyB* mutant plants flowered at about the same time as the wild type (Fig. 4; blue). Consistent with our model, *phyB* mutation can suppress the late-flowering phenotype of cry2 under blue-plus-red light, whereas the cry2 mutation cannot suppress the early-flowering phenotype of *phyB* in red light (26).

Although our model explains the mode of action of cry2 and phyB in the regulation of flowering time of *Arabidopsis*, phyA and cry1 appear to function in different ways in this process (6, 8, 9), and the relative importance of individual photoreceptors in mediating photoperiodic signals may be different in other plant species (9). It will also be interesting to learn the relationship between cry2 in photoperiodism and the circadian clock associated with blue-light-entrained circadian rhythms in plants (27).

REFERENCES AND NOTES

1. R. E. Kendrick and G. H. M. Kronenberg, *Photomorphogenesis in Plants* (Kluwer Academic, Dordrecht, ed. 2, 1994); H. Mohr, *ibid.*, pp. 353–373; H. Smith, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 289 (1995); M. Furuya and E. Schafer, *Trends Plant Sci.* **1**, 301 (1996); A. von Arnim and X.-W. Deng, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 215 (1996).
2. W. Briggs, R. L. Heath, E. Tobin, *Regulation of Plant Growth and Development by Light*, vol. 17 of *Current Topics in Plant Physiology* (American Society of Plant Physiologists, Rockville, MD, 1996).
3. P. H. Quail *et al.*, *Science* **268**, 675 (1995).
4. J. Chory, *Plant Cell* **9**, 1225 (1997).
5. M. Ahmad and A. R. Cashmore, *Nature* **366**, 162 (1993); E. Liscum and R. P. Hangarter, *Plant Cell Env.* **17**, 639 (1994); C. Lin *et al.*, *Science* **269**, 968 (1995); M. Ahmad and A. R. Cashmore, *Plant Mol. Biol.* **30**, 851 (1996); C. Lin and A. R. Cashmore, *in* (2), pp. 30–39.
6. N. Goto, T. Kumagai, M. Koornneef, *Physiol. Plant.* **83**, 209 (1991).
7. J. W. Reed, P. Nagpal, D. S. Poole, M. Furuya, J. Chory, *Plant Cell* **5**, 147 (1993).
8. D. J. Bagnall *et al.*, *Plant Physiol.* **108**, 1495 (1995); D. J. Bagnall, R. W. King, R. P. Hangarter, *Planta* **200**, 278 (1996); R. King and D. Bagnall, *Semin. Cell Dev. Biol.* **7**, 449 (1996); M. T. Zagotta *et al.*, *Plant J.* **10**, 691 (1996).
9. E. Johnson, M. Bradley, N. P. Harberd, G. C. Whitelam, *Plant Physiol.* **105**, 141 (1994); K. J. Haliday, M. Koornneef, G. C. Whitelam, *ibid.* **104**, 1311 (1994); J. L. Weller, I. C. Murfet, J. B. Reid, *ibid.* **114**, 1225 (1997); J. L. Weller, J. B. Reid, S. A. Taylor, I. C. Murfet, *Trends Plant Sci.* **2**, 412 (1997).
10. C. Lin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2686 (1998).
11. The strategy used in the isolation of the cry2 mutant is based on the observation that overexpression of CRY2 resulted in a short hypocotyl under blue light, especially low intensity of blue light (10). Fast-neutron-mutagenized Columbia (Col-4) seeds were sown on compound soil, kept in a cold room (4°C) in the dark for 4 days, exposed to ~150 $\mu\text{mol s}^{-1} \text{m}^{-2}$ of cool white fluorescent light for 1 day, and grown under low intensity of blue light (2 to 5 $\mu\text{mol s}^{-1} \text{m}^{-2}$) for 2 to 3 days. Seedlings that grew taller than average were selected. Individual isolates were analyzed for the CRY2 expression using the immunoblot assay (10). Two cry2 alleles were identified from 636 isolates initially selected from ~130,000 M_2 seeds. Lights and filters used are as described (10).
12. M. Koornneef, C. J. Hanhart, J. H. van der Veen, *Mol. Gen. Genet.* **229**, 57 (1991).
13. J. M. Martinez-Zapater, G. Coupland, C. Dean, M. Koornneef, in *Arabidopsis*, E. M. Meyerowitz and C. R. Somerville, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994), pp. 403–433; R. Simon and G. Coupland, *Semin. Cell Dev. Biol.* **7**, 419 (1996); A. J. M. Peeters and M. Koornneef, *ibid.*, p. 381; K. A. Hicks, A. Sundas, D. R. Meeks-Wagner, *ibid.*, p. 409; M. Aukerman and R. M. Amasino, *ibid.*, p. 427; H. Ma, *Cell* **89**, 821 (1997); M. Koornneef, C. Alonso-Blanco, A. J. M. Peeters, W. Soppe, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, in press.
14. G. Coupland, *Trends Genet.* **11**, 393 (1995).
15. D. Bradley, O. Ratcliffe, C. Vincent, R. Carpenter, E. Coen, *Science* **275**, 80 (1997).
16. G. Redei, *Genetics* **47**, 443 (1962).
17. The BAC clone F19P19 containing the CRY2 gene (<http://pgcc-genome.pw.usda.gov/libraries.html>) is located at the 0846 locus, about 5 centimorgans from the PHYA gene in the recombinant inbred map (<http://cbil.humgen.upenn.edu/~atgc/ATGCUP.html> or http://nasc.nott.ac.uk/new_ri_map.html). FHA was mapped to about 4 centimorgans from the PHYA gene in the classical genetic map (http://mutant.lse.okstate.edu/genepage/classical_map.html) (12). For the complementation test, the flowering time of F_1 progenies of cry2-1 (Col background) \times tha-1 (Ler background) was compared with that of the F_1 progenies of cry2-1 \times Ler and Col \times tha-1.
18. D. S. Hsu *et al.*, *Biochemistry* **35**, 13871 (1996).
19. H.-W. Park, S.-T. Kim, A. Sancar, J. Deisenhofer, *Science* **268**, 1866 (1995).
20. J. Putterill, F. Robson, K. Lee, R. Simon, G. Coupland, *Cell* **80**, 847 (1995).
21. T. Araki and Y. Komeda, *Plant J.* **3**, 231 (1993).
22. R. Simon, M. I. Igeno, G. Coupland, *Nature* **384**, 59 (1996).
23. K. D. Jofuku *et al.*, *Plant Cell* **6**, 1211 (1994).
24. J. A. M. Brown and W. H. Klein, *Plant Physiol.* **47**, 393 (1971).
25. K. Eskins, *Physiol. Plant.* **86**, 439 (1992).
26. T. Mockler and C. Lin, unpublished data.
27. A. J. Millar, M. Straume, J. Chory, N.-H. Chua, S. A. Kay, *Science* **267**, 1163 (1995).
28. M. Aitchitt *et al.*, *Plant Mol. Biol. Rep.* **11**, 317 (1993).
29. Single-letter abbreviations for the amino acid residues are as follows: E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; M, Met; R, Arg; S, Ser; and W, Trp.
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Src Activation in the Induction of Long-Term Potentiation in CA1 Hippocampal Neurons

You Ming Lu, John C. Roder,* Jonathan Davidow, Michael W. Salter

Long-term potentiation (LTP) is an activity-dependent strengthening of synaptic efficacy that is considered to be a model of learning and memory. Protein tyrosine phosphorylation is necessary to induce LTP. Here, induction of LTP in CA1 pyramidal cells of rats was prevented by blocking the tyrosine kinase Src, and Src activity was increased by stimulation producing LTP. Directly activating Src in the postsynaptic neuron enhanced excitatory synaptic responses, occluding LTP. Src-induced enhancement of α -amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptor-mediated synaptic responses required raised intracellular Ca^{2+} and *N*-methyl-D-aspartate (NMDA) receptors. Thus, Src activation is necessary and sufficient for inducing LTP and may function by up-regulating NMDA receptors.

Long-term potentiation is a persistent enhancement in the efficacy of synaptic transmission that has been proposed to be a principal cellular substrate underlying learning

and memory (1). LTP is induced by a cascade of biochemical steps that, for a main form of LTP, occur in the postsynaptic neuron (2). Protein tyrosine phosphorylation is